INVESTIGATION OF POSSIBLE APOCRINE GLAND COMPONENT IN BASAL CELL EPITHELIOMA*

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Many basal cell tumors (epitheliomas) take origin from follicular structure. (Fig. 1). This association of basal cell epithelioma with follicular structure is well supported by the literature on the subject (1, 2, 3, 4). It has been pointed out that these tumors show a predominantly benign disposition and seldom invade the dermis to a level below that reached by the hair follicles. Frequently, the tumor masses will simulate rather accurately follicular structure, and they develop only in those areas in which skin appendages occur. The rare instance in which basal cell epithelioma has been reported on the palmar or plantar surfaces (5), or on the mucous membrane, only serves to emphasize the aberrant character of such growths in such locations.

The relationship between the hair follicle and basal cell epithelioma appears to be of functional as well as developmental character. Within the basal cell tumor masses, it is common to observe areas of partially or completely keratinized cells. In such instances, the tumor cells within the mass show progressive differentiation mimicking the changes occurring in a hair follicle as the basal cell develops into a keratinized hair cell. Occasionally, cells resembling sebaceous gland cells appear within the tumor aggregations, and it appears that differentiation may also proceed in this direction.

In addition to differentiation within the tumor masses simulating hair and sebaceous gland formation, it is common to observe cellular aggregations which follow the pattern and configuration of sweat glands or ducts. The apocrine gland with its duct is the appendage associated with the pilosebaceous complex. Phylogenetically, in the lower mammals, it enjoys a universal distribution. In man, this universal distribution occurs only for a short time during the third and fourth months of fetal life. The apocrine bud then regresses, except in certain specialized areas such as the nipple, axillary and genital regions, and except in rare instances when it persists in aberrant locations.

If basal cell epitheliomas may only develop from primary epithelial germ (pilosebaceous anlage), rather than from pluripotential cells existing in either the epithelial or follicular basal cell layer, the glandular tissue in the tumor, if any morphologic or functional differentiation occurs, should show characteristics common to the apocrine gland. Conversely, if pluripotential cells are the ancestors of basal cell epitheliomas, any functional or developmental characteristics of a gland-like nature, might be of either the eccrine or apocrine type.

This investigation was directed at discovering morphologic, or, by histochemical means, functional indication of gland or duct formation and activity.

Considerable literature has accumulated regarding the histochemical characteristics of the sudoriferous glands. Acid phosphatase (6), alkaline phosphatase (6, 7), lipid deposits (7, 8) and McManus positive material (both glycogen and diastase resistant granules) have been reported in association with both apocrine and eccrine sweat glands (6, 8, 9). Glycogen has been observed in the sweat ducts (8). Iron granules are frequently seen in the apocrine sweat glands (7). A concentration of cholinesterase activity has been reported in association with eccrine, but not with apocrine glands (10). Montagna (11) has reported the presence of succinic dehydrogenase activity in the eccrine, and to a lesser degree, in the apocrine glands and ducts. It was believed that application of similar histologic technics to the tissue of basal cell epitheliomas might reveal characteristic glandular reactions and that this information might further elucidate the composition and histogenesis of basal cell epithelioma.

MATERIALS AND METHODS

The characteristics of basal cell epitheliomas submitted for routine diagnostic examination to the laboratory of the Dermatology Department of the Hospital of the University of Pennsylvania

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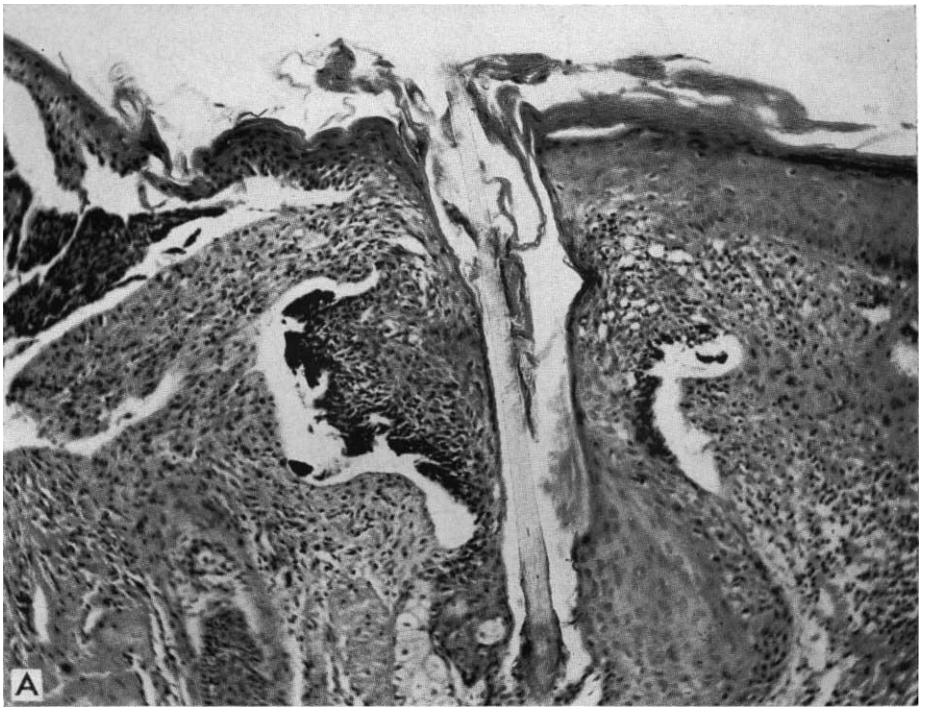

FIG. 1a. Shows origin of basal cell epithelioma from the neck of a hair producing follicle. (120 ×)

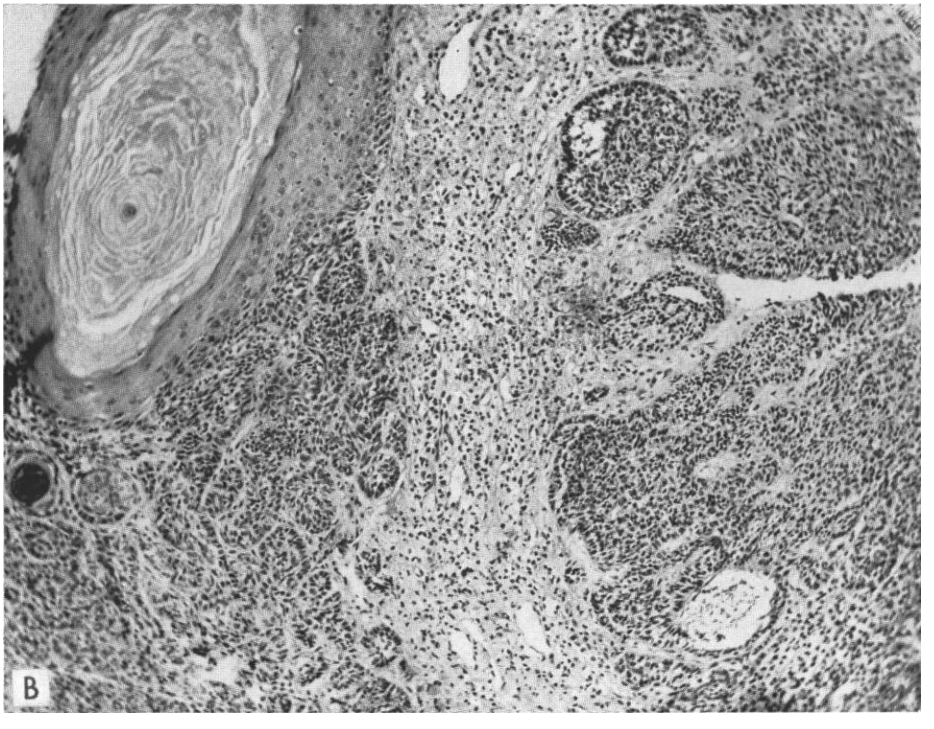

FIG. 1b. Shows basal cell epithelioma originating from the lower portion of a follicle. Also note the degeneration of cells within the tumor aggregations to form the cystic glandular structures in FIG. 3b & c. (120 ×).

during the past 18 months (706 tumors), were reviewed. Of these tumors, 496 were discarded from the study as inadequate specimens because size, fragmentation, or extensive ulceration prohibited accurate orientation and evaluation of the pathologic changes.

Thirty specimens of basal cell epithelioma were initially selected in a random fashion as they were received in the laboratory, eliminating only those specimens too small or too friable and fragmented, and surveyed to determine the distribution of lipids, iron and McManus positive material. The investigation was then extended to 30 tumors which showed some obvious relationship to the pilosebaceous complex. These were selected on the basis of their demonstration on routine examination of (1) continuity of the tumor, with hair producing follicles; (2), evidence of keratinization and/or sebaceous gland differentiation; and (3), formations mimicking glandular or ductal structure. Thirty tumors were also selected for study on the basis of the presence of pigment within the tumor cells.

Formalin fixed, paraffin embedded tissue was submitted to the Prussian blue reaction for iron and to the McManus reaction (12) for detection of mucopolysaccharides. Formalin fixed and fresh non-fixed tissue was frozen, sectioned at 10 to 15 micra, and then stained with sudanophilic dyes for lipids, by the Gomori technic for alkaline phosphatase (13) and by the Koelle technic (14) for localization of cholinesterase activity. Sections of frozen tissue, previously fixed for short periods in acetone, were also stained for acid phosphatase. (Gomori technic (15)).

RESULTS

Basal cell epithelioma originates from the epidermis in about ⅓ of the biopsy specimens submitted to the laboratory for routine diagnostic examination. This includes the superficial type of basal cell epithelioma. Many of the lesions, while demonstrating deep penetration of the dermis by the tumor cells, also show areas indistinguishable from those of superficial type of basal cell epithelioma. Three blocks of tissue showing direct epidermal origin were sectioned serially and, as has been reported by others (4,

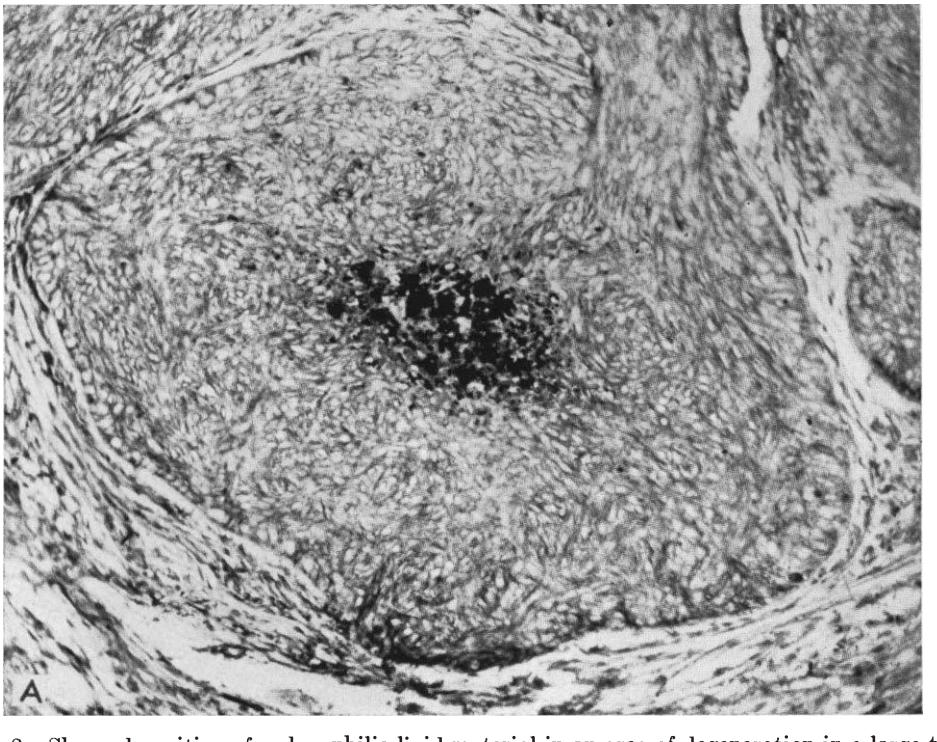

FIG. 2a. Shows deposition of sudanophilic lipid material in an area of degeneration in a large tumor mass. (× 170).

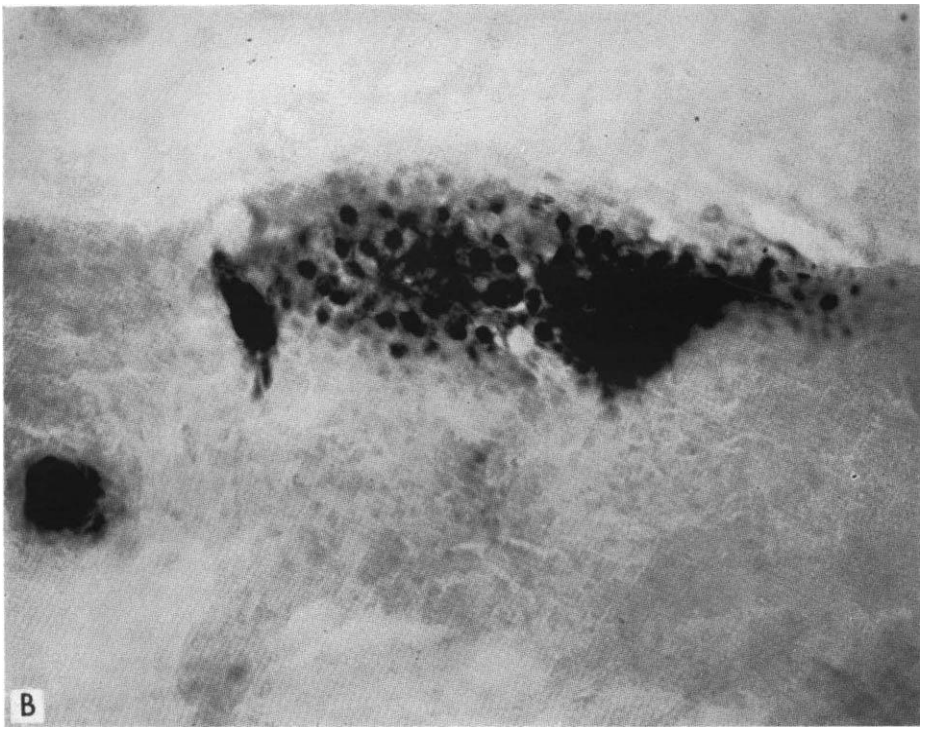

FIG. 2b. Shows alkaline phosphatase activity in cells at the periphery of a tumor mass. Note the concentration of alkaline phosphatase in the lumen of the vessel which curves around the area. (× 260).

16), no relationship between the tumor and hair producing follicles could be demonstrated. No further investigation of this type of tumor was undertaken.

Examination of those basal cell epitheliomas apparently originating from follicular structure revealed no characteristic site or foci of cells along the wall of the hair follicle which could be identified as a consistent source of tumor cells. The tumors arise anywhere along the length of the hair follicle wall. In some instances a single stalk-like attachment is observed, and in others, the attachment extends in a continuous, almost sessile fashion, over a large area of the hair fol licle (Fig. 1.)

Iron pigment was found in only three specimens. In no instance was it found in the tumor cells, but rather in cells within the inflammatory reaction surrounding the tumor.

Lipid deposits were present in considerable quantity within the superficial type of basal cell epithelioma and in areas within the large, massive, space-taking tumor aggregations. These could often be correlated with areas which showed evidence of keratinization or changes suggestive of the holocrine cellular disintegration associated with sebaceous gland activity (Fig. 2a). There was no deposition of lipid material in the formations which mimicked the sudoriferous glands or their ducts, although the stroma surrounding these formations frequently was fairly heavily infiltrated by lipid material.

The McManus positive material associated with basal cell epithelioma varied in amount directly with the presence of either ulceration or inflammation. The cells of the tubular and cystic structures were remarkably free of this material, although the surrounding stroma often consisted of fibers which showed a strongly positive McManus reaction and similarly stained material was frequently observed within the lumen of the cystic structures. A McManus positive membrane lined the luminal surface of the epithelial cells in most instances. This material was resistant to amylase digestion. Intracellular McManus positive, amylase digestible material (glycogen) was demonstrable in the larger tumor masses and in tumor aggregations in proximity to areas of surface ulceration.

Cholinesterase studies (4 tumors) indicated no

direct relationship of positively reacting fibers to the tumor cells. Well preserved nerve fibers are present in the stroma between the tumor masses. An obvious increase in number was occasionally noted. This is apparently due to concentration of the fibers as the stroma is disrupted by the invading tumor masses. Thus, cholinesterase fibers were most conspicuous in the stroma in those areas characterized by the presence of massive, space-taking aggregations of tumor tissue. Fibers were few in number and found only with difficulty in the stroma surrounding the more delicate invading strands of the adenocystic type of basal cell epithelioma.

Alkaline phosphatase activity (10 cases) in the tumor cells was of the same order as could be demonstrated in normal, or relatively normal epithelium at the periphery of the tumor sections. Moderate activity, demonstrated by increased nuclear staining was present in the peripheral cells of tumor masses associated with inflammation and increased vascularity of the surrounding tissue (Fig. 2b). No distinctive staining was observed in the adenoid-like formations.

Acid phosphatase activity was not demonstrated either in the uninvolved epidermis or in the tumor formations. Several groups of sweat glands were present in the deeper portions of the specimens examined; these showed faint activity of the luminal portion of the glands. No such activity was observed in the cells of the tubular and cystic formations.

Through the courtesy of Dr. George Hambrick, we have had the opportunity of examining one case of basal cell epithelioma stained for the demonstration of succinic dehydrogenase activity. The staining of the associated tumor cells did not approximate the brilliant coloration of the eccrine sweat tubules.

DISCUSSION

Krompecher (17), in the first detailed histologic study of basal cell epithelioma (1900), referred to the tumor as "carcinoma eptheliale adenoides". The literature since that report is replete with descriptions and allusions to the adenoid, tubulo-cystic patterning of many of the tumors, Lever (4) states that in basal cell epithelioma, "the tubular structures are regarded as apocrine, rather than as eccrine sweat gland structures".

Morphologic study is unfortunately liable to subjective interpretation and it is interesting to note that Lennox and Wells (18) in their excellent survey and study on differentiation in basal cell tumors found only one tumor which "showed reasonably convincing duct-like structures" and further state that "No other tumor showed any structure even remotely resembling a sweat gland".

We found two types of cystic structures which might be construed to represent cross sections of glands or ducts. The first (Fig. 3a) is formed by sheets of epithelium which invade and partially or wholly surround or engulf islands of dermal tissue and although continuous in some planes, in section appear in a tubular or cystic pattern. The associated stroma is edematous and composed of diastase resistant McManus positive material. A concentration of this material is seen on both surfaces of the tubular and cystic formations. The presence of a McManus positive "basement membrane" on the luminal surface of the cystic structures would seem strong evidence against this structure being either morphologically or functionally a gland.

The second type of cystic structure (Fig. 3b) was found in only two of the tumors examined. In both instances they were located deep in the tumor. Morphologically, the size is comparable with that of dilated apocrine glands. At least a portion of the wall is lined with a row of cuboidal cells with an outer layer of flatter cells suggestive of myoepithelial cells. Small cytoplasmic buds can be seen protruding into the lumen, further mimicking the appearance of apocrine glands (Fig. 3c). McManus positive material is present in the stroma and forms a "membrane" along the external surface of the cyst. No McManus positive material was found within the lumen, or along the luminal surface of the epithelial cells.

There is thus some morphologic evidence that these cystic structures may represent apocrine gland differentiation. However, no evidence of substantiating functional activity can be offered. Glycogen and iron could not be identified in the paraffin sections and no fresh tissue was available to permit examination for lipids, or for phosphatase or succinic dehydrogenase activity. Further, solid aggregations of cells of similar size and shape in other areas of the tumor showed various degrees of disintegration of the inner mass of cells (Fig. 1b). The peripheral or

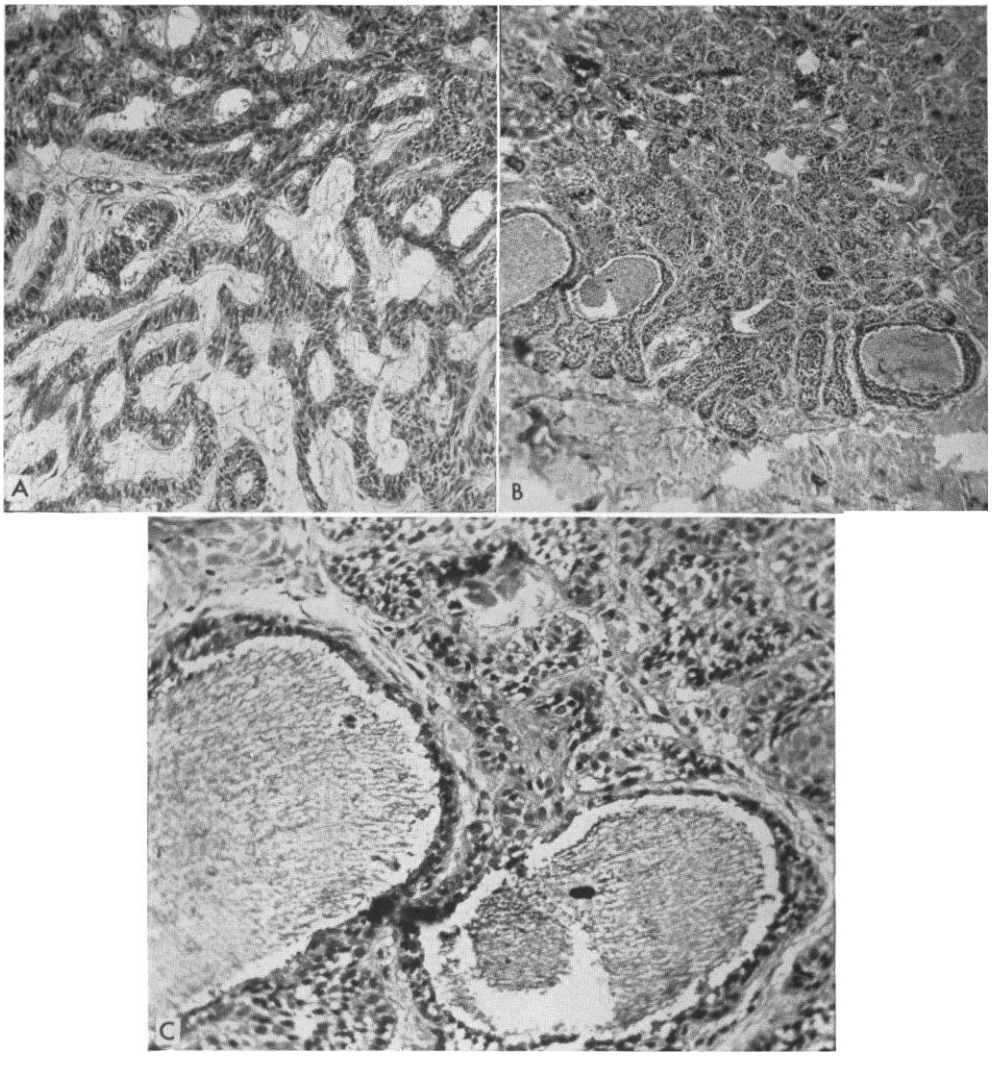

FIG. 3a. Shows the common tubulo-cystic arrangement found in basal cell epithelioma (× 116). FIG. 3b shows the more rare type of tubulo-cystic formation (× 52.5). FIG. 3c. Shows the double row of cells and cytoplasmic buds simulating apocrine gland structure (× 205).

palisade layer of cells appears well preserved and this would seem a likely mode of formation for this particular type of cyst. It, of course, may be noted that gland formation is accomplished by a similar initial production of a solid tubular mass of cells which later develop a lumen, presumably by degeneration of the more centrally placed cells.

The concept advanced by Foot in 1951 (19) that basal cell epithelioma arises from the neck of the hair follicle is an attractive one. Consistent origin of the tumor cells from an area associated with the origin of the apocrine gland, would certainly be a firm foundation for the presence of apocrine gland differentiation in basal cell epithelioma. While in several instances, we were able to demonstrate a tumor connecting with the neck of the follicle (Fig. 1a), it was much more often the case that the tumor connection was with the deeper portions of the follicle (Fig. 1b). In some instances, the follicles involved were of the lanugo type, poorly developed or apparently in a resting stage. It is entirely possible that some of the structures

which we identified as hair follicles could be interpreted as attenuated follicular buds or partially differentiated primary epithelial germs.

Finally, no evidence was found of functional activity which could be unequivocably (or even tentatively) described as characteristic of either eccrine or apocrine glands.

SUMMARY

1. The piliary system, the pilosebaceous anlage or primary epithelial germ often have been described as the source of basal cell epithelioma. Glandular differentiation in tumors of such origin should follow the pattern and characteristics of the apocrine gland. Examination of 210 basal cell tumors has been undertaken to determine either functional or morphologic evidence of an apocrine gland component in basal cell epithelioma.

2. Examination of the cells of basal cell epithelioma for alkaline and acid phosphatase activity, and for deposits of glycogen, lipoid and iron revealed no positive evidence of functional activity which could be attributed to a glandular component in these tumors.

3. It is extremely rare (2 in 210 cases) to find cellular formations within basal cell epitheliomas, which by morphology and polarity of the cells answer the criteria of glandular structure.

4. The possibility of an apocrine gland component in basal cell epithelioma is not eliminated. However, except for the two instances in which apocrine gland morphology was fairly convincingly demonstrable, no positive evidence of apocrine gland participation in the formation of basal cell epithelioma was found.

We wish to express our appreciation to Dr. George Koelle for assistance with the cholinesterase preparations. Photomicrographs were taken by Mr. Edward Glifort.

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DISCUSSION

Dr. Herbert Mescon (Boston, Mass.): This is an excellent, thorough review of the histochemistry of these tumors. I think it should, once and for all, put the death knell on the need for trying to subdivide, subclassify as to point of origin or histologic appearance even of the different variants of tumors that arise from the basal cell layer, be they the basal cell layer of

the sweat gland, the sweat duct, the sebaceous gland, the apocrine gland, the hair follicle, or the basal layer of the surface epithelium. All the experimental work has tended to show that these are pluripotential cells and the continued subclassification, except for clinical purposes, should now be completely discarded.

DR. IAN O. STAHLE (Melbourne, Australia): I believe the writers of this paper have used histochemical investigations to the limit.

In keeping with your plea this morning for adequate correlation between clinical and investigative work, I would make a plea from the clinical side and say, yes, we cannot determine the nature of these tumors any further at the histochemical level but let us go back to the clinical side and I would like to quote just one case of a basal cell carcinoma variant in which a lady, middle aged with a basal cell tumor on the side of the neck could produce bullae, which at times would be hemorrhagic at the premenstrual level. Therefore we may gain more knowledge of these conditions by observing such details as color changes and contour changes and adequately recorded these, especially if we find these tumors in females with a menstrual cycle.

DR. HERMANN PINKUS (Monroe, Michigan): This was a most interesting paper and I would like to agree with Dr. Mescon that this study really supports the point of view that there is not much point in trying to derive basal cell tumors from any specific structure in the skin. It is preferable to be satisfied with the statement Dr. Wood made in the beginning that all these tumors come from pluripotential ectodermal cells. In my opinion, the conclusion that the exact histogenesis of an individual tumor in the skin is of little importance as long as we are sure it comes from ectodermal epithelium of the skin; this conclusion is the major step forward that has resulted from various anatomical, pathological, and experimental studies during the past few years.

I would like to make one more point. If one sets out to look for the origin of any tumor, basal cell or anything else, he can do this with any kind of assurance only if he is dealing with a microscopic, early stage of such a tumor. As soon as a tumor has grown to clinical size I don't feel capable any more of saying that it originated from a hair follicle because it is connected with the follicle, or from a gland because it is connected with the gland. It may very well have grown together with that structure. Certainly a basal cell epithelioma of one millimeter size has been growing for several months and secondary connections may have been established. In this sense we can be very grateful to Dr. Wood and her collaborators to have done this painstaking study and to have brought us these negative conclusions.

Dr. CHARLES WOLF (New York, N. Y.): I would like to commend the wonderful piece of work by the Department of Dermatology, School of Medicine, University of Pennsylvania, and particularly Dr. Wood's presentation.

I believe all those who practice clinical dermatology have been often confused by a certain epithelioma repeatedly seen and treated, responds favorably while on the other hand the same type of epithelioma occurring in other patients will not respond, will be obdurate in treatment, will recur. I believe a point which is to be brought out phylogenetically to the effect that perhaps these so-called similar-appearing cells under the microscope are not so similar. There is that phylogenetic point where they originate from, whether from the basal cell layer, whether from the pilosebaceous system or from the vestigial apocrine gland which makes the difference.

I believe in the future if we do fail with the form of treatment that we pursue we must consider that the reason for it might be in this phylogenetic characteristic.

DR. FRANCIS A. ELLIS (Baltimore, Md.): I enjoyed the paper very much. I was just wondering what percentage of the tumors were pigmented. If some are pigmented it would mean that they arose from the surface epidermis, or the hair follicle and not from either the sebaceous glands, or sweat glands.

The other interesting point is if the basal cell epitheliomas arise from the surface epidermis, why are so relatively few of them pigmented, or why are any of them pigmented? In other words, when basal cell epitheliomas arise from surface epithelium, the dentritic cells (pigmented cells or melanocytes) usually do not take part. Why should any of the basal cell epitheliomas be pigmented?

DR. MARGARET GRAY WOOD (in closing): I thank the discussers.

Dr. Pinkus' point about the fallacy of drawing conclusions from the morphologic observation of a tumor at any specific moment is one that we are aware of only too keenly. Some tumors showed connections with the epidermis, with both epidermis and hair follicles, and some with the hair follicle alone. This caused us to form this broad base, in that our only criterion for selection was the connection of the tumor with the hair follicle. It seemed probable that if an apocrine gland component existed, it would be in those tumors which showed morphologic continuity with follicular structure.

As far as pigmented tumors are concerned, we did select a group of 30 tumors which contained pigment. Iron stains, McManus stains and lipid stains revealed no extraordinary findings. They contained no iron. The pigment is, as we all know, melanin. We saw lipids within these tumors in a considerably greater amount than we did in other types of tumors, with the exception of the superficial basal cell epitheliomas which contain lipids in practically the same amounts as in the pigmented basal cell tumors. The McManus stain showed no excess of glycogen.